Control of Enterotoxic *Bacillus cereus* on Poultry or Red Meats and in Beef Gravy by Gamma Irradiation

ABSTRACT

The gamma-radiation resistance of five enterotoxic and one emetic isolate of *Bacillus cereus* vegetative cells and endospores was tested in mechanically deboned chicken meat (MDCM), ground turkey breast, ground beef round, ground pork loin and beef gravy. The D_{10} values for *B. cereus* ATCC 33018 were 0.184, 0.431 and 2.56 kGy for logarithmic-phase cells, stationary-phase cells, and endospores at 5°C on MDCM, respectively. Neither the presence nor absence of air during irradiation significantly affected radiation resistance of vegetative cells or endospores of *B. cereus* ATCC 33018 when present on MDCM. Irradiation temperature (-20 to +20°C) did affect the radiation resistance of stationary-phase vegetative cells and to a limited extent that of spores on MDCM. Impedance studies indicated that surviving vegetative cells were severely injured by radiation. A dose of 7.5 kGy at 5°C was required to eliminate a challenge of 4.6×10^3 *B. cereus* ATCC 33018 from temperature-abused MDCM (24 h at 30°C). The radiation resistance of a mixture of endospores of six strains to gamma radiation was 2.78 kGy in ground beef round, ground pork loin and beef gravy, but 1.91 kGy in turkey and MDCM. The results indicate that irradiation of meat or poultry can provide significant protection from vegetative cells but not from endospores of *B. cereus*.

Key Words: *Bacillus cereus*, poultry, red meats, gravy, gamma irradiation

---

Bacillus cereus has been implicated in many outbreaks of food poisoning (*8,22*). The implicated foods include poultry, meat and meat products, grains, shellfish, milk products and dried ingredients, such as herbs and spices (*1,2,9,10,14,18,23-25*). There were 112 food-poisoning incidents associated with *Bacillus* species between 1975 and 1984, of which 13 were associated with meat or poultry (*25*). In Japan, 18.3% of meat products and 6.6% of raw meat were contaminated with *B. cereus* (*14*). Quantitative data for *B. cereus* on meat or meat products is rare; however, Sooltan et al. (*23*) found 1.4 to 3.5 colony forming units (CFU)/g on retail chicken and turkey products in Bristol, United Kingdom.

Reference to a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Investigators paid relatively little attention to the effects of ionizing radiation on *B. cereus* associated with meat and almost none to the effects of radiation on the vegetative cell rather than on the endospore. Ma and Maxcy (*15*) reported that the endospore was more resistant at ambient temperature than at -30°C and that its resistance was not affected to the same extent as were vegetative cells of other genera by changes in temperature, drying or suspending medium. The actual effects of gamma radiation on the vegetative cells of *B. cereus* were not directly investigated. The radiation dose required to inactivate 90% (D_{10} value) of the late stationary-phase vegetative cells of *B. cereus* NCTC 11145, a diarrheal strain, on roast beef and in gravy were 0.173 and 0.181 kGy (1 Gy = 100 rad), respectively, at 3 to 4°C (*12*). A 2 kGy gamma-radiation dose decreased growth and toxin production by *B. cereus* NCTC 11145 in roast beef and gravy at abuse temperatures of 15 and 22°C (*13*). The results of a study of the effects of sub-sterilization gamma-radiation doses on the survival and injury of *B. cereus* ATCC 33018 on mechanically deboned chicken meat and of a mixture of five enterotoxic strains and one emetic *B. cereus* strain on mechanically deboned chicken meat, lean ground beef, ground pork loin, ground turkey breast and gravy are presented in this report.

MATERIALS AND METHODS

Culture and growth conditions.

The following diarrheal strains of *B. cereus* were maintained and cloned on tryptic soy agar (TSA; Difco Laboratories, Detroit, MI) and incubated at 30°C: ATCC 33018, B4AC(2), F4433/73, Watertown, F45814/70 and the emetic strain F4552/75. The ATCC 33018 was obtained from the American Type Culture Collection (ATCC) and is an enterotoxic strain isolated from powdered milk-base formula (*26*). The remaining strains were obtained from the culture collection at the Eastern Regional Research Center and were described by Buchanan and Schultz (*3*). Culture identity was confirmed by gram stain, endospore formation and from reactions on the *Bacillus* card of the Vitek AMS Automicrobic System (bioMérieux Vitek, Inc., Hazelwood, MO). All cultures were identified as *B. cereus* except for the Watertown and F4552/75 isolates, which were identified as *Bacillus thuringiensis*. Both of these strains tested negative for amylase. Since both strains produced food poisoning typical of *B. cereus* we regard them as

being *B. cereus*. Tryptic soy broth (100 ml) (TSB; Difco) in a 500 ml baffled Erlenmeyer culture flask was inoculated with 1 ml of TSB from a 15 to 18 h culture incubated at 30°C. These cultures were incubated with shaking (150 rpm) at 30°C for 3 h (logarithmic-phase cells) or for 16 h (stationary-phase cells). These culture conditions were determined to be appropriate by preliminary studies. A 10-fold concentrated inoculum was commonly prepared by centrifuging (1,725 × *g* at 5°C for 30 min) and resuspending the cells in one-tenth volume of Butterfield's phosphate (0.25 m KH_2PO_4 adjusted to pH 7.2 with NaOH) (Fisher Scientific Co., Pittsburgh, PA).

Spore suspensions were prepared according to the method of Dornbush and Abbey (*4*). Briefly, growth was washed from a mature slant and used to inoculate the surface of 300 ml of the following medium: Bacto peptone (Difco), 3.5 g; Bacto nutrient broth (Difco), 4 g; dextrose, 1 g; Bacto agar, 15 g; per 1 L distilled water. The pH was adjusted to pH 6.55. The inoculum was spread evenly over the agar and incubated for 1 week at 30°C. The cells were washed from the agar with approximately 50 ml of sterile Butterfield's phosphate. The suspension was heat shocked at 65°C for 30 min and washed three times with sterile distilled water, centrifuging between each washing. The suspension was then heat shocked for a second time at 70°C for 30 min and stored at refrigeration temperatures.

Substrates, packaging conditions and proximate analyses.

Mechanically deboned chicken meat consisting of approximately 90% rib and 10% back meat was obtained in 18-kg lots from a commercial manufacturer of poultry frankfurters. Lean ground round of beef, lean ground pork loin and ground turkey were obtained from a local grocery store. A commercial dehydrated brown gravy mix was prepared according to the manufacturer's instructions and sterilized by autoclaving. The meat substrates were subdivided into 100 ± 0.1 g amounts and then spread thinly and vacuum-sealed in Stomacher 400 (Tekmar Co., Cincinnati, OH) polyethylene bags. These bags were themselves vacuum sealed in Freshstuff oxygen barrier pouches (oxygen transmission 0.6 to 0.8 cm^3/645 cm^2/24 h at 3.2°C and 90% relative humidity) (American National Can Company, Des Moines, IA) to provide better protection during handling and to prevent oxygen transmission to the samples. The meat was frozen at -50°C and sterilized by gamma irradiation to a dose of 42 kGy at -30°C. Both sterile and non-sterile meat were stored at -20°C until use.

Proximate analyses were performed in duplicate by a U.S. Department of Agriculture (USDA) certified commercial laboratory. The total reductive capacity of each of the substrates (gravy, beef, pork, turkey and MDCM) was determined in duplicate by titration with 0.002 m 2,6-dichlorophenol-indophenol sodium salt (*7*).

Radiation source and irradiation techniques.

The self-contained gamma-radiation source of ^{137}Cs had a strength of approximately 134,000 Ci (4.95 PBq) and a dose rate of 0.114 kGy min^{-1}. The dose rate was established using National Physical Laboratory (Middlesex, U.K.) dosimeters. Variations in absorbed dose were minimized by placing thin samples (approximately 2 mm) within a uniform portion of the radiation field. The total sample mass irradiated at one time usually did not exceed 20 g. Samples were maintained within ± 0.5°C of the desired temperature by injecting the gas phase from liquid nitrogen into the irradiation chamber. Sample temperature was monitored continuously during irradiation.

Inoculation of meat for determination of D_{10} values.

Sterile meat was mixed well with an average of $10^{8.6}$ stationary-phase CFU/g, $10^{5.8}$ log-phase CFU/g, or $10^{5.4}$ endospores/g of *B. cereus* suspended in Butterfield's phosphate buffer. Samples of 5.0 ± 0.05 g of the inoculated meat were aseptically transferred to sterile Number 400 polyethylene Stomacher™ bags. Mixing was accomplished by stomaching the sample for 90 s using a Stomacher 400 (Tekmar). The inoculated meat was spread uniformly over an area of about 10 × 10 cm within the bag and heat sealed either *in vacuo* or with air trapped within the bag, as appropriate. Each bag was then vacuum-packaged within a Freshstuff™ bag to prevent oxygen absorption by vacuum-packed samples and to provide additional microbiological security for all samples during irradiation and subsequent handling.

Determination of D_{10} values.

Sterile MDCM, inoculated with stationary-phase CFU of *B. cereus* ATCC 33018, received radiation doses of 0 to 3.0 kGy in increments of 0.5 kGy at 5°C. The meat was packaged either *in vacuo* or with air in the package. The study was replicated four times.

Sterile MDCM, inoculated with mid-log-phase CFU of *B. cereus*, received radiation doses of 0 to 1.05 kGy in increments of 0.15 kGy at 5°C. The meat was packaged either *in vacuo* or with air in the package. The study was replicated three times.

Sterile MDCM, inoculated with *B. cereus* endospores, received radiation doses of 0 to 4.0 kGy in increments of 0.5 kGy at 5°C. The meat was packaged either *in vacuo* or with air in the package. The study was replicated twice.

Appropriate radiation doses were identified by preliminary studies to provide a minimum of five measurements within the linear portion of the survival curve.

Effect of temperature during irradiation treatment.

A modified central composite response-surface design (*5*) was used to determine the effect of irradiation temperature (-20 to +20°C) on the survival of stationary-phase CFU of *B. cereus* ATCC 33018 in vacuum-packaged sterile ground beef. The inoculation (2.32×10^8 CFU/g) and packaging procedures were identical to those used to determine D_{10} values. Two replicate samples were analyzed for each of the following combinations of irradiation temperature and dose: -20°C, 0, 1.5, 3.0 kGy; -10°C, 0, 0.75, 2.25 kGy; 0°C, 0, 3.0 kGy; +10°C, 0, 0.75, 2.25 kGy; +20°C, 0, 1.5, 3.0 kGy. Five replicate samples treated at 0°C with 1.5 kGy were analyzed.

The survival of heat shocked *B. cereus* spore suspensions of ATCC 33018, B4AC(2), F4433/73, Watertown, F45814/70 and F4552/75 in 5.0 ± 0.05 g of MDCM after a radiation dose of 3.0 kGy *in vacuo* at temperatures of 10, 5, 0, -5, -10, -20, -30, -40, -50 and -60°C was measured by determining the number of CFU as described below. The study was replicated twice.

Challenge and temperature-abuse study.

Sterile MDCM was inoculated with endospores of *B. cereus* ATCC 33018 or a mixture of stationary-phase cells of all six strains. Samples of 5.0 ± 0.05 g were vacuum-packaged in sterile Stomacher bags and then themselves vacuum sealed in American National Can Company Freshstuff bags. One set of the 5-g samples was analyzed immediately following irradiation at the indicated doses at 5°C for total surviving CFU. The second set of 5-g samples was temperature abused at 30°C for 20 h before analysis. Before temperature abuse each sample bag was opened and expanded to provide oxygen for the growth of the culture. This study was repeated twice. Some samples containing stationary-phase cells from all six strains were heated for 30 min at 70°C after irradiation but before temperature abuse.

Microbiological analysis.

Samples were assayed for CFU by standard pour-plate procedures using TSA with serial dilutions in sterile Butterfield's phosphate. Petri plates were incubated for 24 h at 30°C. Colony

forming units were counted on three petri plates having 30 to 300 colonies with a New Brunswick Scientific Biotran II automated colony counter. Preliminary studies indicated that maximal CFU were obtained within 24-h incubation.

Impedance analysis of cell injury.

Impedance analyses were performed using the bioMérieux Vitek Bactometer. The number of surviving CFU was determined for each sample. Samples (1.0 ml) for assay of detection time were withdrawn from the 10^{-2} dilution for plate counting and mixed with 9 ml of Wilkins-Chalgren Anaerobe broth (Oxoid, Ltd., Basingstoke, Hampshire, England). Portions (2.0 ml) were placed in two assay wells of the Bactometer plate to determine detection time at 30°C. Standard curves were prepared for detection by plotting impedance detection times against the measured estimates of the viable CFU for uninjured (non-irradiated) cells prepared from serial dilutions of the unirradiated control samples in each study. The population of the irradiated samples can be estimated for each impedance detection time from the standard curve, or vice versa. The difference between this value and that obtained from counts of CFU on TSA pour plates represents the injured population. The assumption is that injured cells require more time to initiate the log-growth phase than do uninjured cells, as found by Mackey and Derrick (*16*). Because detection time depends on both the initial number of cells per unit volume and the lag time (*17*), we do not equate the detection time with lag time. Mackey and Derrick used conductance measurements to estimate the lag phase of injured *Salmonella typhimurium* (*17*).

Statistical analysis.

Radiation D_{10} values were determined by least squares analysis of the survival data, excluding the 0 kGy data to avoid possible shoulder effects, using the regression procedure of the Statistical Analysis System (SAS) statistical package (*8,19*). Regression techniques were used to fit second-order response-surface models (*5*), and calculations were performed using the general linear models procedure of the SAS statistical package (*8*). The regressions were tested for differences by analysis of covariance.

RESULTS

Substrates

The results of proximate analyses of the substrates are presented in Table 1. The estimates of the reductive capacity per gram of each of the substrates were 2.8, 19.6, 17.6, 8.2 and 7.8 mm for gravy, beef, pork, turkey and MDCM, respectively. These results are interpreted to mean that beef and pork contain more reducing substances than do the poultry meats. The assumption is that at least the sulfhydryl portion of these reducing substances might serve as competitive scavengers for radiation-induced free radicals.

TABLE 1. *Proximate analyses of substrate composition.*

Substrate	% Fat	% Moisture	% Protein	% Ash
MDCM[a]	21.9	64.2	16.3	0.97
Turkey	5.95	74.3	18.6	1.15
Beef	6.25	70.2	21.8	1.25
Pork	12.9	65.8	21.4	1.35
Gravy	0.65	92.4	0.95	1.4

[a] Mechanically deboned chicken meat.
All values are the result of the analysis of two independent samples.

Radiation D_{10} values.

Analysis of covariance revealed that the presence or absence of air during irradiation of logarithmic-phase cells, stationary-phase cells, and endospores of *B. cereus* in MDCM did not significantly alter the gamma-radiation D_{10} values for *B. cereus* ATCC 33018. Thus, the raw data for both aerobic and vacuum-packed samples were combined and D_{10} values were derived for each of the three sets of data (Table 2, Fig. 1). The logarithmic-phase cells were 2.1 times as sensitive to gamma radiation as were the stationary-phase cells and 12 times as sensitive as were the endospores. The stationary-phase cell was 5.7 times as sensitive to gamma radiation as was the *B. cereus* endospore (Table 2).

Effect of temperature during irradiation.

Vegetative cells of *B. cereus* ATCC 33018 were not significantly ($p = 0.0725$) affected by temperatures between -20 to ± 20°C; however, when the vegetative cells were irradiated, the interaction between radiation dose (kGy) and temperature was significant ($p = 0.0204$) (Fig. 2). The Type I probabilities for the sources of variation from the analysis of variance were 0.0001, 0.0725, 0.0204, 0.4032 and 0.1866 for kGy, temperature, kGy × temperature, kGy^2 and $temperature^2$, respectively. The equation developed from the analysis of variance predicting the response of *B. cereus* ATCC 33018 to irradiation at temperatures from -20 to +20°C is the following:

$$\text{LOG SURVIVORS} = -0.1275 - 1.3657 \times \text{kGy} + 0.0079 \times \text{temperature} - 0.0153 \times \text{kGy} \times \text{temperature} - 0.1169 \times \text{kGy}^2 + 0.0008 \times \text{temperature}^2$$

This equation predicts that *B. cereus* irradiated with 3.0 kGy in MDCM at -20, 0 and +20°C would decrease in number by 4.20, 5.27 and 5.72 log units, respectively.

TABLE 2. *Gamma radiation D_{10} values for* B. cereus *ATCC 33018 logarithmic and stationary-phase vegetative cells and endospores suspended in mechanically deboned chicken meat at 5°C.*

Growth phase	Packaging atmosphere[a]	D_{10} value (kGy) ± S.E.[b]	N[c]	R-Square
Logarithmic	Air	0.180 ± 0.012	15	0.942
Logarithmic	Vacuum	0.187 ± 0.011	15	0.956
Logarithmic	Air & vacuum	0.184 ± 0.008	30	0.946
Stationary	Air	0.417 ± 0.023	24	0.936
Stationary	Vacuum	0.446 ± 0.019	24	0.962
Stationary	Air & vacuum	0.431 ± 0.016	48	0.944
Endospore	Air	2.46 ± 0.309	12	0.863
Endospore	Vacuum	2.67 ± 0.075	12	0.946
Endospore	Air & vacuum	2.56 ± 0.216	24	0.864

[a] Atmosphere present in package during irradiation. Air and vacuum indicates that the reported value resulted from the combination of results from both atmospheres.
[b] S.E.: standard error for determination.
[c] Number of estimates for logarithm_{10} of the *B. cereus* CFU surviving treatment with gamma radiation used in the calculation of the D_{10} value.

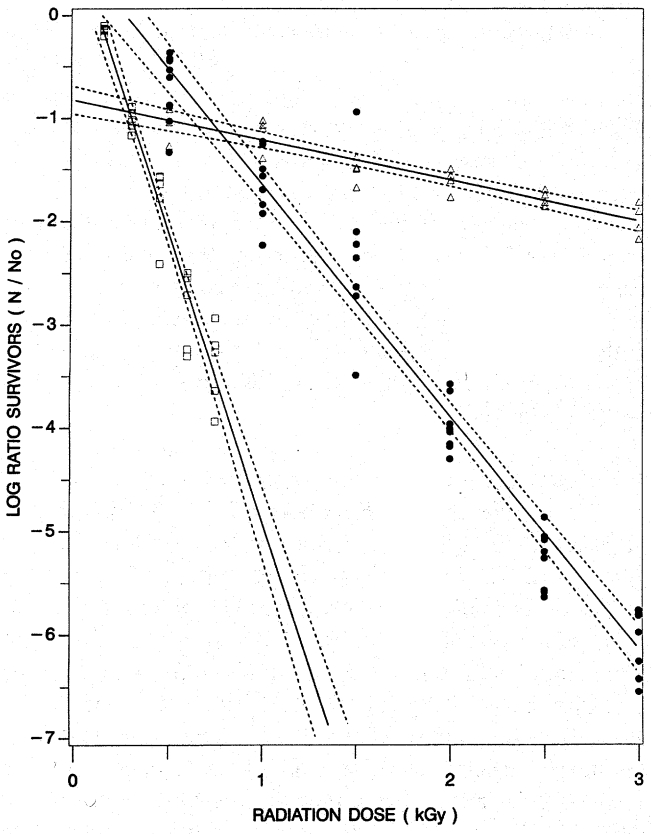

Figure 1. *Gamma radiation survival curves on mechanically deboned chicken meat for* B. cereus *ATCC 33018 logarithmic-phase vegetative cells (❑) (grown for 3 h in TSB), stationary-phase vegetative cells (●) (16 to 18 h in TSB), and endospores (Δ). The dashed lines represent the 95% confidence limits for the regressions.*

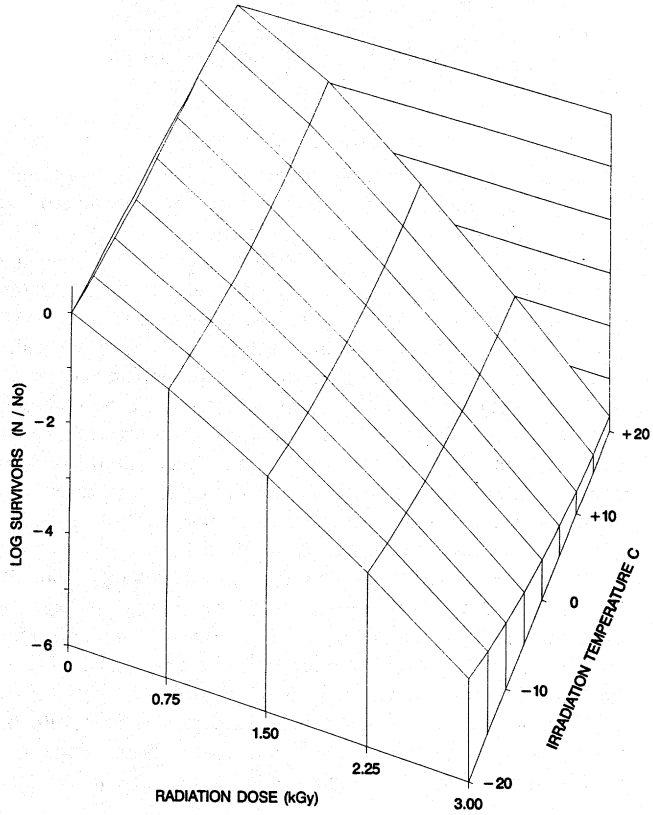

Figure 2. *Predicted survival of stationary-phase CFU of* B. cereus *ATCC 33018 in vacuum-packaged ground beef irradiated at temperatures between -20 and +20°C.*

The reduction in the number of viable *B. cereus* spores on MDCM by a radiation dose of 3.0 kGy was not greatly affected by irradiation temperature. There were no significant differences in response between the irradiation temperatures of + 10 to 0°C or again between -10 to -60°C. The measured log reductions in the number of surviving *B. cereus* at irradiation temperatures of +10 to 0°C, -5°C and -10 to -60°C were 0.96 ± 0.07, 0.75 ± 0.09, and 0.56 ± 0.08, respectively.

Challenge and temperature-abuse study.

An inoculum of 4.6×10^3 CFU/g of *B. cereus* ATCC 33018 on refrigerated MDCM was lowered to 260 CFU/g by 1.5 kGy (Table 3). The number of CFU in the temperature-abused samples indicates that a dose of 7.5 kGy is required to completely inactivate a challenge of 4.6×10^3 endospores of *B. cereus* ATCC 33018 per g on MDCM.

Shamsuzzaman (*20*) found that spores of *C. sporogenes* irradiated at 5.5 kGy in phosphate buffer and then heated were much more sensitive to heat than the unirradiated spores or those irradiated after heating. A similar increased sensitivity to heat was found for irradiated *Clostridium perfringens* endospores by Gombas and Gomez (*11*). A radiation dose of 3.0 kGy decreased the detectable CFU from a mixture of stationary-phase cells from six strains of *B. cereus* from 3.72 log units on MDCM to 1.11, but if the meat was heated to 70°C for 30 min after irradiation 0.82 CFU were detected (Table 4). In this study all temperature-abused samples had prolific pathogen multiplication and could be expected to be toxic. Because prolific growth occurred in the heat-shocked samples during temperature abuse there is no evidence that vegetative cells or endospores of *B. cereus* surviving treatment with gamma radiation were more sensitive to heat than non-irradiated cells or endospores.

Radiation injury to vegetative cells.

The extent of radiation injury can be estimated by comparing the measured impedance detection times to the predicted impedance detection times for equivalent populations of uninjured vegetative cells of *B. cereus* ATCC 33018. The predicted impedance detection times were derived from a standard curve for non-irradiated populations relating impedance detection times to population as described in "Materials and Methods." The increased impedance detection time for the irradiated cell is related to increased lag time for the injured cell (*16,17*). The time required to detect logarithmic-phase cells was significantly different from the expected detection time (Fig. 3). Similar results were obtained with stationary-phase cells (Fig. 4). The populations represented in Fig. 2 and Fig. 3 are the same as those presented in the survival curves in Fig. 1 for logarithmic-phase and stationary-phase cells. The percentage of injured versus non-injured cells can be estimated by comparing the actual population to that calculated from the detection time. Thus, it was discovered that the injury to log-phase cells was directly related to dose, and that

TABLE 3. *Survival of* B. cereus *ATCC 33018 endospores on gamma irradiated mechanically deboned chicken meat with and without temperature abuse.*

Temperature abuse	Radiation dose (kGy)						
	0	1.5	3.0	4.5	6.0	7.5	9.0
	CFU						
None[a]	4.6×10^3	2.6×10^2	32	2	0	ND[b]	ND
20 h at 30°C	8.9×10^7	2.3×10^7	7.5×10^6	2.0×10^5	5.0×10^3	ND	ND

[a] The non-abused samples were analyzed immediately.
[b] Not detected (lower limit of detection 10 CFU/g).
These results are the means of two independent studies.

TABLE 4. *Survival of a mixture of six strains[a] of* B. cereus *stationary-phase[b] cells on irradiated MDCM with and without heating at 70°C and/or temperature abuse.*

Storage			Logarithm$_{10}$CFU/g[c]					
Temp.	Hours	Heated[d]	0 kGy	1.0 kGy	2.0 kGy	3.0 kGy	4.0 kGy	5.0 kGy
5°C	0	No	3.72	1.61	1.83	1.11	1.06	ND[e]
5°C	0	Yes	ND	ND	0.52	0.82	ND	ND
30°C	20	No	8.40	7.65	6.50	6.54	6.24	5.74
30°C	20	Yes	7.32	8.04	6.65	6.98	3.92	6.95

[a] *Bacillus cereus* ATCC 33018, Watertown, F4552/75, 45814/70, B 4AC(2) and F4433/73 challenge at 3.72 log CFU/g.
[b] 16-h cultures at 30°C.
[c] Means of three independent studies.
[d] Samples were heated for 30 min at 70°C.
[e] Not detected.

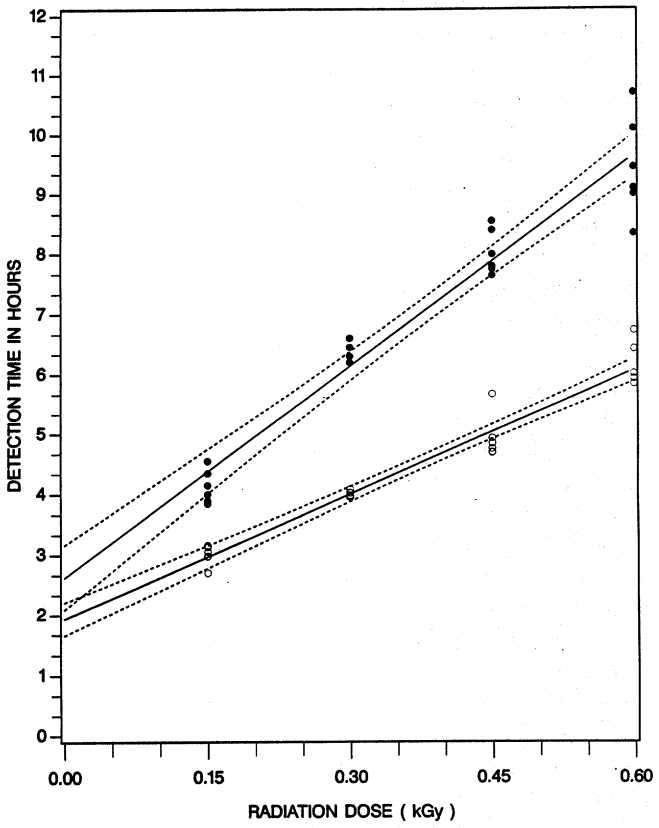

Figure 3. *Impedance detection times (●) and predicted impedance detection times (○) for irradiated* B. cereus *ATCC 33018 logarithmic-phase cells. The dashed lines represent the 95% confidence limits for the regressions.*

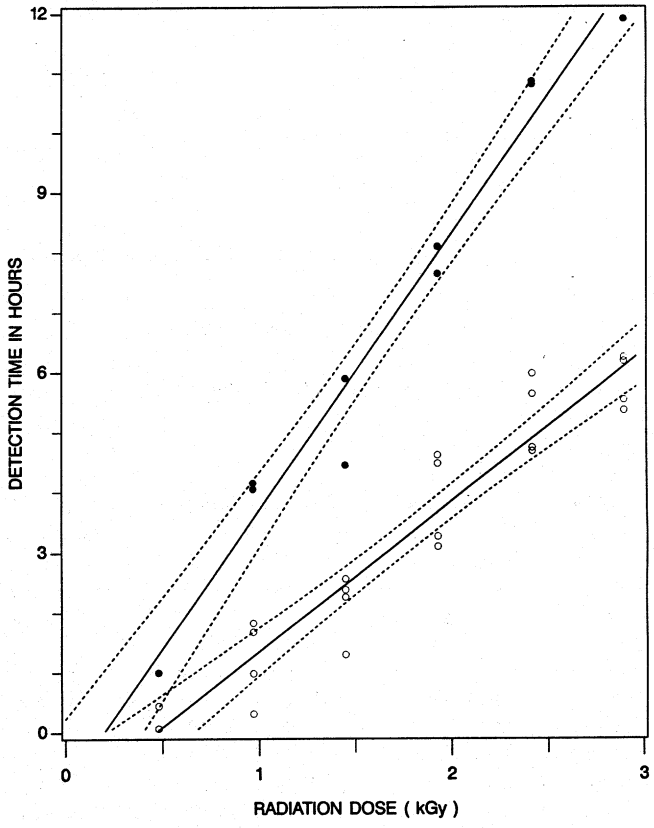

Figure 4. *Impedance detection times (●) and predicted impedance detection times (○) for irradiated* B. cereus *ATCC 33018 stationary-phase cells. The dashed lines represent the 95% confidence limits for the regressions.*

0.6 kGy injured approximately 95% of the cells (Fig. 5). The injury to stationary-phase cells was also directly related to dose, and approximately 70% of the cells were injured by 2.0 kGy.

The radiation resistance of a mixture of the endospores of six strains of *B. cereus* did not differ significantly ($p > 0.05$) when irradiated on beef, pork, and in gravy (Table 5). The radiation resistance of the mixture of endospores did not differ when they were irradiated on turkey or MDCM (Table 5). The radiation resistance of this mixture of endospores was significantly ($p < 0.003$) less on MDCM or turkey than when they were on beef and pork, or in gravy. Comparison of the proximate analyses for the substrates did not explain the differences in radiation resistance of *B. cereus* endospores (Table 1). For example, beef and turkey were low in fat and had similar values for protein and moisture. Yet the resistance of the endospore mixture was identical in pork and beef gravy, which had very dissimilar proximate analyses. Fox et al. (*7*) reported that the different rates of radiolytic destruction of thiamin in red meats and chicken breast could be accounted for, in part, by the different levels of natural reductants in these substrates. The reductive capacity of the meat substrates does seem to correspond to the radiation resistance of the endospore, but radiation resistance of endospores in gravy did not follow the same pattern. The gravy mix contained citric acid, BHA and propyl gallate as preservatives. Though these preservatives would have been present in very small quantities, it is possible that they may account for the unexpectedly high D_{10} value of the endospore in this gravy. The effect of oxygen is often cited to explain different results in various substrates. This is not the case in this study because all samples were vacuum packaged and then repackaged in a barrier bag prior to irradiation. Thus, the oxygen tension should have been low during irradiation in all samples. Additional research will be required to explain the differing responses of *B. cereus* endospores to gamma radiation in different substrates.

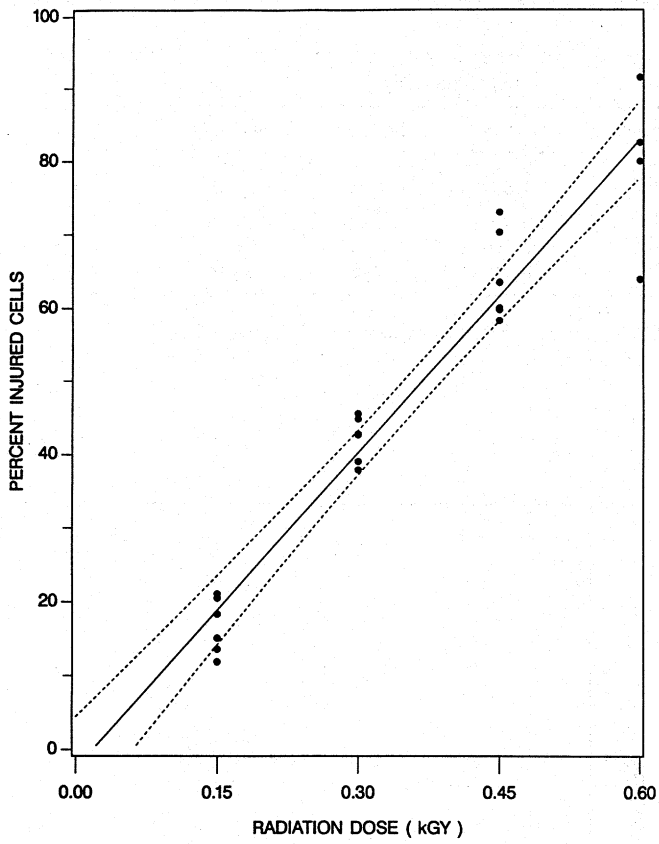

Figure 5. *Percent injured log-phase vegetative cells of* B. cereus *ATCC 33018 after irradiation.*

TABLE 5. *Comparison of gamma-radiation D_{10}-values[a] at 5°C for a mixture of six strains[b] of* B. cereus *vacuum-packaged in various substrates.*

Growth phase	Substrate	D_{10}-value (kGy) ± SE	N	R-Square
Stationary	MDCM	0.619 ± 0.013	12	0.996
Endospore	MDCM	1.92 ± 0.072	24	0.970
Endospore	Beef round	2.78 ± 0.173	24	0.921
Endospore	Pork loin	2.78 ± 0.315	24	0.780
Endospore	Turkey breast	1.90 ± 0.113	24	0.928
Endospore	Beef gravy	2.77 ± 0.205	24	0.892

[a] Means of three independent studies.

[b] *Bacillus cereus* ATCC 33018, Watertown, F4552/75, 45814/70, B 4AC(2) and F4433/73.

DISCUSSION AND CONCLUSIONS

The D_{10} value for *B. cereus* stationary-phase cells in chicken meat was 0.45 ± 0.02 kGy at 5°C with no significant differences between survival on MDCM packed *in vacuo* rather than with air. Logarithmic-phase vegetative cells were more sensitive to gamma radiation (0.18 ± 0.01 kGy) than were stationary-phase cells. Presumably the greater sensitivity derives from the rapid replication of deoxyribonucleic acid (DNA) and other cell components during the logarithmic phase of growth (*21,22*). Neither of these values is grossly dissimilar to the D_{10} values for other foodborne pathogens, such as *Salmonella*. Both the vegetative cells and the endospores of *B. cereus* were significantly more resistant to gamma radiation when the meat was frozen during irradiation. These results differ from those obtained by Ma and Maxcy (*15*) who reported that *B. cereus* endospores were more sensitive to radiation at sub-freezing temperatures. Our finding that irradiated *B. cereus* vegetative cells and spores were less sensitive at reduced temperatures is similar to results reported for other vegetative cells and for spores of *Clostridium botulinum* (*6*). The results reported here are also compatible with theory as the mobility of the hydroxyl radical is decreased at sub-freezing temperatures and in products, such as spores where the water content is reduced. Endospores were significantly more resistant to gamma radiation than were vegetative cells (D_{10} value >2 kGy). Impedance measurements of detection times indicated that surviving vegetative cells were severely injured by radiation. A dose of 7.5 kGy was required to eliminate a challenge of 4.6×10^3 endospores of *B. cereus* from mechanically deboned chicken meat that was irradiated at 5°C and then abused at 30°C for 20 h.

Grant and Patterson (*12*) reported D_{10} values of 0.173 and 0.181 kGy at 3 to 4°C for late exponential-phase cells of *B. cereus* NCTC 11145 on roast beef and in gravy, respectively. Our result of 0.184 kGy for the D_{10} value of logarithmic-phase cells of *B. cereus* ATCC 33018 on MDCM is not significantly different. The current study demonstrated that the stationary-phase vegetative cell is significantly more resistant to ionizing radiation than the logarithmic-phase vegetative cell. Controlling stationary-phase cells and endospores may be more important for food safety than controlling logarithmic-phase cells because of the high radiation resistance of the endospore and because the stationary phase would probably be reached in most abused foods.

Several organizations in the United States have expressed interest in the irradiation of red meats. This would require amending the current Food and Drug Administration approval for control of foodborne pathogens on poultry to include red meats. Such an action by the Food and Drug Administration will necessarily be based on data on the radiation-sensitivity of various foodborne pathogens on red meats and poultry under similar conditions of irradiation. To provide information that might be useful for such a petition to the Food and Drug Administration we investigated the gamma-radiation resistance of a mixture of endospores of six strains of enterotoxic *B. cereus* on MDCM, ground beef round, ground pork loin, ground turkey breast and in beef gravy and found that the radiation resistance of the endospores was significantly lower on poultry meat than on red meats.

The mean of the D_{10} values for the endospores on beef or pork was 2.78 kGy and is considerably less than that for *C. botulinum* (3.4 to 3.6 kGy) on cooked beef at 25°C (*27*). The endospores of *C. sporogenes* had a D_{10} value of 3.04 to 3.52 kGy when irradiated at 0°C in either nutrient broth or 0.067 m sodium phosphate (pH 7.0) (*20*).

The results indicate that significant protection from vegetative cells will be granted to the consumer by irradiating poultry or red meats to a maximum dose of 3.0 kGy but not if heat shocked endospores are present. The results also indicate that radiation resistance values for *B. cereus* on one meat substrate should not be extrapolated to include other untested substrates.

ACKNOWLEDGMENT

Portions of this manuscript were presented at the International Food Technology Exposition & Conference, The Hague, The Netherlands, November 15-18, 1992. The authors thank J. D. Fox, Jr., N. Fromer, H. Jugueta, E. Kobylinski, B. Mueller, C. Paquette and K. Snipes for their technical assistance and B. L. Bowles, E. A. Johnson and S. C. Thayer for their review of the manuscript.

REFERENCES

1. Bean, N. H. and P. M. Griffin. 1990. Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, vehicles and trends. J. Food Prot. 53:804-817.
2. Bryan, F. L. 1980. Foodborne diseases in the United States associated with meat and poultry. J. Food Prot. 43:140-150.
3. Buchanan, R. L. and F. J. Schultz. 1992. Evaluation of the Oxoid BCET-RPLA Kit for the detection of *Bacillus cereus* diarrheal enterotoxin as compared to cell culture cytotoxicity. J. Food Prot. 55:440-443.
4. Dornbush, A. C. and A. Abbey. 1972. Microbiological assay of the tetracyclines. pp. 365-383. *In* F. Kavanagh (ed.). Analytical Microbiology. Vol. II. Academic Press. New York, NY.
5. Draper, N. R. and H. Smith. 1981. Applied regression analysis. 2nd ed. John Wiley & Sons, Inc., New York, NY.
6. El-Bisi, H. M., O. P. Snyder and R. E. Levin. 1966. Radiation death kinetics of *C. botulinum* spores at cryogenic temperatures. pp. 89-106. *In* M. Ingram and T. A. Roberts (eds.). Botulism 1966, Proc. 5th Int. Symp. Food Microbiol., Moscow. Chapman and Hall, Ltd., London.
7. Fox, J. B., Jr., L. Lakritz and D. W. Thayer. 1993. The effect of reductant level in skeletal muscle and liver on the rate of loss of thiamin due to gamma radiation. Int. J. Radiat. Biol. (In press).
8. Freund, R. J., R. C. Littell and P. C. Spector. 1986. Statistical Analysis System for linear models. SAS Institute, Inc., Cary, NC.
9. Gilbert, R. J. 1979. *Bacillus cereus* gastroenteritis. pp. 495-518. *In* H. Riemann and F. L. Bryan (eds.). Foodborne Infections and Intoxications. 2nd ed. Academic Press, New York, NY.
10. Goepfert, J. M., W. M. Spira and H. U. Kim. 1972. *Bacillus cereus*: Food poisoning organism: A review. J. Milk Food Technol. 35:213-227.
11. Gombas, D. E. and R. F. Gomez. 1978. Sensitization of *Clostridium perfringens* spores to heat by gamma radiation. Appl. Environ. Microbiol. 36:403-407.
12. Grant, I. R. and M. F. Patterson. 1992. Sensitivity of foodborne pathogens to irradiation in the components of a chilled ready meal. Food Microbiol. 9:95-103.
13. Grant, I. R., C. R. Nixon and M. F. Patterson. 1993. Effect of low-dose irradiation on growth of and toxin production by *Staphylococcus aureus* and *Bacillus cereus* in roast beef and gravy. Int. J. Food Microbiol. 18:25-36.
14. Konuma, H., K. Shinagwa, M. Tokumaru, Y. Onoue, S. Konno, N. Fujino, T. Shigehisa, H. Kurata, Y. Kuwabara and C. A. M. Lopes. 1988. Occurrence of *Bacillus cereus* in meat products, raw meat and meat product additives. J. Food Prot. 51:324-326.
15. Ma, K. and R. B. Maxcy. 1981. Factors influencing radiation resistance of vegetative bacteria and spores associated with radappertization of meat. J. Food Sci. 46:612-616.
16. Mackey, B. M. and C. M. Derrick. 1982. The effect of sublethal injury by heating, freezing, drying and gamma-radiation on the duration of the lag phase of *Salmonella typhimurium*. J. Appl. Bacteriol. 53:243-251.
17. Mackey, B. M. and C. M. Derrick. 1984. Conductance measurements of the lag phase of injured *Salmonella typhimurium*. J. Appl. Bacteriol. 57:299-308.
18. Van Netten, P., A. Van de Mossdijk, P. Van Hoensel, D. A. A. Mossel and I. Perales. 1990. Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. J. Appl. Bacteriol. 69:73-79.
19. Statistical Analysis System Institute, Inc. 1987. SAS-STAT guide for personal computers, Version 6. SAS Institute, Inc., Cary, NC.
20. Shamsuzzaman, K. 1988. Effects of combined heat and radiation on the survival of *Clostridium sporogenes*. Radiat. Phys. Chem. 31:187-193.
21. Singh, H. and J. A. Vadasz. 1983. Effect of gamma radiation on *E. coli* ribosomes, tRNA and aminoacyl-tRNA synthetases. Int. J. Radiat. Biol. 43:587-597.
22. Stapleton, G. E. 1955. Variations in the sensitivity of *Escherichia coli* to ionizing radiations during the growth cycle. J. Bacteriol. 70:357-362.
23. Sooltan, J. R. A., G. C. Mead and A. P. Norris. 1987. Incidence and growth potential of *Bacillus cereus* in poultry meat products. Food Microbiol. 4:347-351.
24. Ternstrom, A. and G. Molin. 1987. Incidence of potential pathogens on raw pork, beef and chicken in Sweden, with special reference to *Ersipelothrix rhusiopathiae*. J. Food Prot. 50:141-146.
25. Todd, E. C. D. 1992. Foodborne disease in Canada — a 10-year summary from 1975 to 1984. J. Food Prot. 55:123-132.
26. Torres-Anjel, M. J., C. Basile, H. P. Riemann and L. V. Butcher. 1980. Suggested epidemiology of *Bacillus cereus* and other spore forming organisms in mishandled breast milk substitutes. Proc. World Congr. Foodborne Infect. Intox., Berlin, Germany. pp. 298-305.
27. Urbain, W. M. 1986. Food irradiation. Academic Press, Inc., New York, NY.